An engineered glycoprotein inhibitor of cholera toxin

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Introduction

Protein-carbohydrate interactions at cell surfaces mediate many important processes in biology from fertilisation to adhesion of viruses, bacteria and their toxins. Individually, protein-sugar interactions are usually very weak, but both affinity and binding selectivity can be enhanced through a phenomenon called multivalency: multiple binding sites on the protein interact simultaneously with multiple copies of the sugar ligand to achieve a high avidity and enhance binding selectivity. A good example of this class of proteins is the cholera toxin which binds to five copies of a specific glycolipid ligand on the surface of cells that line the intestine. Binding to the cell surface leads to internalisation of the toxin, therefore, inhibitors of the protein-sugar interaction have the potential to be used as anti-toxin drugs.

We have developed methods to make chemically defined glycoprotein inhibitors that are matched in size and valency to the target toxin. The cholera toxin B-subunit was first converted to a non-binding mutant, to which the carbohydrate ligands were appended selectively at the N-termini of the pentameric protein by an oxime ligation reaction. The glycoprotein inhibitor was around 14,000 times more potent as an inhibitor than the monovalent carbohydrate. A combination of dynamic light scattering and analytical ultracentrifugation demonstrated that the inhibitor formed 1:1 complexes with the target toxin. The methods developed in this project have the potential to provide a general strategy for making inhibitors against multimeric proteins.

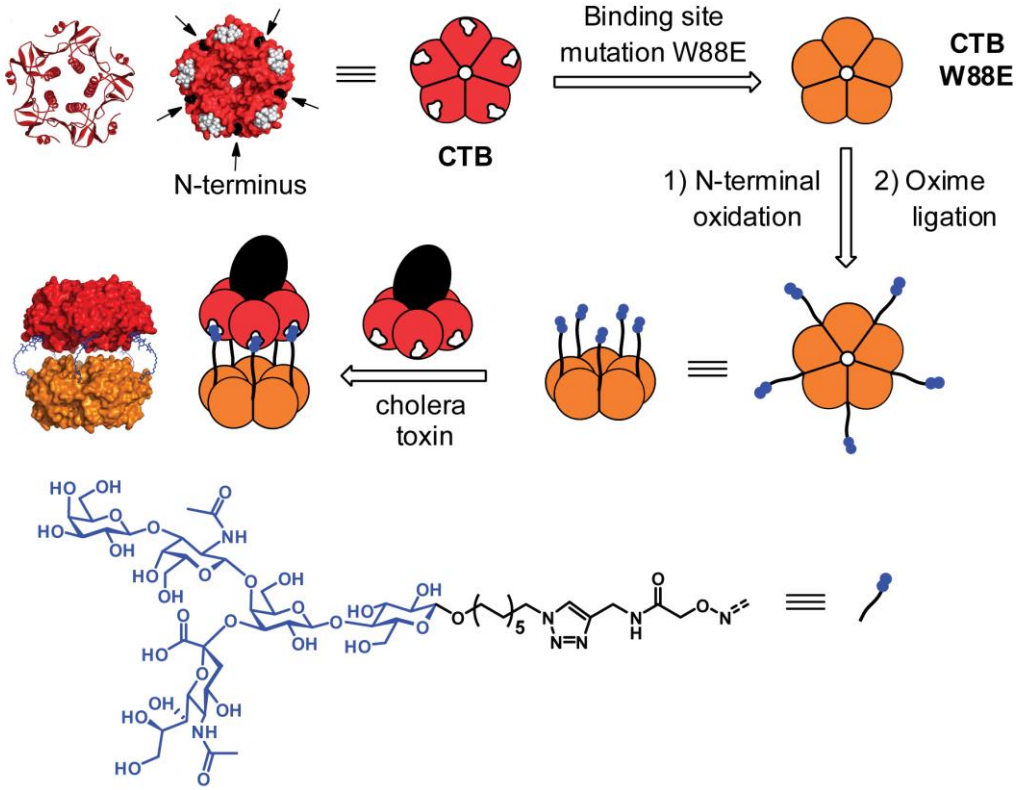

Figure 1: Re-engineering the cholera toxin B-subunit by site-directed mutagenesis and N-terminal oxidation and oxime ligation. Part of the figure is reproduced with permission from Branson et al., (2014) *Angew. Chem. Int. Ed*. **53**, 8323-8327.

Publications

Branson, T., Mcallister, T., Garcia-Hartjes, J., Fascione, M., Ross, J., Warriner, S., Wennekes, T., Zuilhof, H. & Turnbull, W. (2014) A protein-based pentavalent inhibitor of the cholera toxin B-subunit. *Angew. Chem. Int. Ed. Engl.* **53**: 8323-8327.

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